INFLUENCE OF TRYPTOPHAN ON THE ACTIVITY OF ACETYLCHOLINESTERASE IN THE BRAIN OF WELL-FED NORMAL AND ADRENALECTOMIZED RATS

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SUMMARY

Eight hours after a single tube-feeding of tryptophan the activity of acetylcholinesterase in the cerebral hemisphere of well-fed (non-fasted) normal and adrenalectomized rats was 28 and 53% higher, respectively, compared to the corresponding water-fed control. On the other hand, the enzyme activity in the cerebellum of both normal and adrenalectomized rats remained essentially unchanged following tryptophan administration. Pretreatment of adrenalectomized rats with actinomycin-D totally abolished the tryptophan-mediated stimulation of cerebral acetylcholinesterase activity. The pattern of response of cerebral acetylcholinesterase in well-fed adrenalectomized rats over a period of 24-hr following a dose of tryptophan was found to be biphasic.

Recently we have demonstrated that a single tube-feeding of tryptophan to both well-fed adrenalectomized and adrenalectomized-diabetic rats markedly stimulates amino acid incorporation into a number of hepatic proteins *in vivo* (1-3). Hepatic ribosomes isolated from tryptophan-treated adrenalectomized rats also show a higher capacity to synthesize proteins *in vitro* (4). Furthermore, we have observed that whereas tryptophan stimulates the level of hepatic microsomal cytochrome P-450, and aniline hydroxylase activity in well-fed normal rats, it does not do so in the well-fed adrenalectomized rats (5), indicating that all hepatic proteins are not equally affected by tryptophan. This, together with our more recent observation that tryptophan stimulates amino acid incorporation

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into total brain protein in well-fed adrenalectomized rats (2), prompted us to study the activity of enzymes in the brain of normal and adrenalectomized rats after a dose of tryptophan. In the present investigation the activity of acetylcholinesterase in the cerebral hemisphere and cerebellum of well-fed (non-fasted) normal and adrenalectomizedrats was measured following a single tube-feeding of tryptophan.

MATERIALS AND METHODS

Chemicals. Acetylthiocholine iodide, and 5,5'Dithio(bis) dinitrobenzoic acid were obtained from Boehringer Mannheim, West Germany. Actinomycin-D was a product of Calbiochem, La Jolla, California, U.S.A. All other chemicals were the same as reported earlier (5).

Animals and treatment. Male Wistar rats (250 - 300 g) were supplied with ad. lib. commercial laboratory diet throughout the experimental period. Bilateral adrenalectomy was done 6 days before use, and were treated the same way as described earlier (1, 2). On the day of the experiment, rats were given by a stomach tube either L-tryptophan (30 mg/100 g) in distilled water or an equivalent volume of water and killed at different time intervals. The animals were killed by decapitation, and the brains were rapidly removed. The cerebral hemisphere and cerebellum were dissected out, and were frozen immediately on solid CO_2.

Assay of acetylcholinesterase. The frozen tissues were partially thawed and weighed. A 2% homogenate (w/v) was prepared in 0.1 M phosphate buffer, pH 8.0, and 100 µl aliquots were assayed for acetylcholinesterase activity by the method of Ellman et al. (6) at 37°C with acetylthiocholine as substrate on an Unicam SP-800 spectrophotometer.

RESULTS AND DISCUSSION

The activity of acetylcholinesterase in the brain has been found to change in response to a number of chemical and physical treatments (7 - 10). In the first series of experiments the responsiveness of brain acetylcholinesterase to tryptophan force-feeding was studied in both well-fed normal and adrenalectomized rats. The results presented in the Table-1 reveal that after eight hours of tryptophan force-feeding the activity of acetylcholinesterase in the cerebral

TABLE 1

Effect of tryptophan force-feeding on the activity of acetylcholinesterase in the cerebral hemisphere and cerebellum of well-fed normal and adrenalectomized rats.

Treatment	Acetylcholinesterase activity (moles/l.per min × 10^{-6} per g of tissue)			
	Normal		Adrenalectomized	
	Cerebral hemisphere	Cerebellum	Cerebral hemisphere	Cerebellum
Water-fed (Control)	15.5 ± 0.99 (100)	5.0 ± 0.94 (100)	14.1 ± 0.78 (100)	4.4 ± 0.29 (100)
Tryptophan	19.8 ± 1.18 (128; P < 0.05)	5.3 ± 0.42 (106; N.S)	21.6 ± 1.89 (153; P < 0.01)	5.0 ± 0.43 (114; N.S)

Intact normal and adrenalectomized rats were killed 8 hr after tryptophan or water-treatment. Each value represents the mean ± S.E.M. of 5-10 determinations from three rats. The values in the parentheses represent percentage of the respective water-fed control as well as P value. N.S = non significant.

hemisphere of normal and adrenalectomized rats was 28 and 53% increased, respectively, as compared to the corresponding water-fed control. Although the enzyme activity in the cerebellum of both normal and adrenalectomized rats showed slight increments (6 - 14%) following tryptophan treatment, the values were not found to be significantly different when compared with the respective water-fed control (Table-1), indicating that acetylcholinesterase of different regions of the brain do not respond similarly to tryptophan stimulus.

Our present observation that tryptophan stimulates acetylcholinesterase activity in both intact normal and adre-

nalectomized rats is different from that noted earlier for hepatic microsomal cytochrome P-450, the activity of which is stimulated by tryptophan in normal rats but not in adrenalectomized rats (5). One possible explanation for the difference in responsiveness of these two enzymes in adrenalectomized rats to tryptophan stimulus would be that whereas tryptophan-mediated stimulation of brain acetylcholinesterase activity is independent of adrenal hormone(s), the presence of it is required for the activation of hepatic microsomal cytochrome P-450. In support of this,we have observed that adrenalectomy by itself does not significantly lower the acetylcholinesterase activity in the brain (Table-1), while a considerable reduction of hepatic microsomal cytochrome P-450 occurs after bilateral adrenalectomy (5).

In the next series of experiments the time-interrelationship of the activity of acetylcholinesterases in the cerebral hemisphere and cerebellum of adrenalectomized rats was studied following a single tube-feeding of tryptophan. The pattern of response for acetylcholinesterase of cerebral hemisphere over a period of 24-hr following a dose of tryptophan was found to be biphasic. Thirty minutes after tryptophan administration the activity of the enzyme in the cerebral hemisphere was found to be 55% higher than the control, which declined slowly over the next 4.5 hr, and then increased again (Fig-1). Such an observation is similar to that found earlier for hepatic protein and RNA synthesis in well-fed adrenalectomized rats after tryptophan administration. However, the activity of acetylcholinesterase in the cerebellum did not follow any definite pattern,though remained slightly elevated over that of the control upto 12 hr after tryptophan treatment (Fig-1).

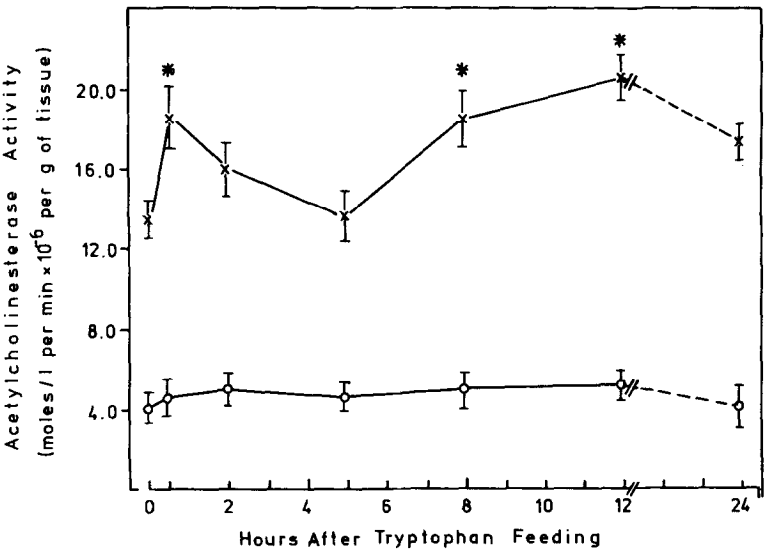

Fig. 1. Effect of a single tube-feeding of tryptophan to well-fed adrenalectomized rats on the activity of acetylcholinesterase in the cerebral hemisphere and cerebellum regions of the brain. Comparison with water-fed control. The rats were killed 0.5, 2, 5, 8, 12 and 24 hr after tryptophan administration. The water-fed controls (zero-time) were killed 0.5 hr after administration of water. The cerebral hemisphere (x——x), and cerebellum (o——o) were assayed for acetylcholinesterase activity. Each value on the curve represents the mean ± S.E.M. of 4-6 determinations using 2 rats.
* Significantly different compared to the water-fed control either at a level of $P < 0.05$ or lower as judged by students "t" test.

Recently we have demonstrated that in well-fed adrenalectomized rats tryptophan enhances amino acid incorporation into proteins of liver, brain and kidneys (2). Furthermore, we have also shown that, at least with respect to liver protein synthesis, an acute effect of tryptophan could only be seen when RNA synthesis is unimpaired (2). To determine whether RNA synthesis is required for the tryptophan-mediated stimulation of cerebral acetylcholinesterase activity, the adrenalectomized rats were injected with actinomycin-D or saline 30 min prior to administration of either tryptophan

TABLE 2

Influence of actinomycin-D on the activity of acetylcholinesterase in the cerebral hemisphere of adrenalectomized rats, fed either water or tryptophan

Treatment	Acetylcholinesterase activity (Moles/l. per min $\times 10^{-6}$ per g of tissue)
Water-fed (Control)	15.1 ± 1.56 (100%)
Tryptophan	24.4 ± 1.92 (162; $P < 0.01$)
Actinomycin-D + water	14.2 ± 0.62 (94; N.S)
Actinomycin-D + tryptophan	15.4 ± 1.11 (102; N.S)

Actinomycin-D (100 µg/100 g) was injected intraperitonally 30 min before administration of water or tryptophan, and killed 8.5 hr later. Each value represents the mean ± S.E.M. of 6 determinations from two rats.

or water. In the saline-treated rats administration of tryptophan caused a 62% higher enzyme activity when compared with water-fed controls (Table-2). However, in the antibiotic-treated rats acetylcholinesterase activity in the cerebral hemisphere, measured 8 hr after tryptophan force-feeding was found to be the same as that of the control (Table-2). The results clearly indicate that an increased RNA synthesis is associated with the tryptophan-mediated stimulation of acetylcholinesterase activity in the well-fed adrenalectomized rats. Therefore, the attainment of an enhanced activity of acetylcholinesterase in the cerebral hemisphere of

the adrenalectomized rats after tryptophan administration can be interpreted as due to an increased synthesis of enzyme per se.

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